GENERAL CONSIDERATION

Reproductive functions in vertebrate species are essentially a consequence of neuroendocrine activities. An invariant principle component of the neuroendocrine conglomerate which regulates reproductive activities, is the Hypothalamo-Hypophysial-Gonadal axis, represented by the releasing factors of the hypothalamus, the gonadotrophic hormones of the pituitary and the gonadal steroids. Since organisms have to adapt and adjust to their particular habitat and the annual variations in the environmental conditions, a multifactorial regulation of the reproductive axis seems to have evolved to ensure reproductive success. These factors could be either extrinsic such as light, heat, temperature, humidity and pheromones or intrinsic which may include endocrine secretions, neurotransmitters, nutritional factors and gonadal autocrine and paracrine factors. These regulatory factors seem to have developed the ability to influence reproductive functions by modulating the reproductive axis at different levels. Such factors are of great relevance in seasonal breeders and the extended degree of involvement vary from species to species and depends on the compulsions of a given species. However, the involvement of these factors has been secondarily minimised in continous breeders due to the acquisition of constant and optimal environmental conditions due to either domestication or a

hormones

sheltered life. A survey of existing literature on the existing reproductive functions in vertebrates, reveals the involvement of many hormones like prolactin, pituitary hormones, adrenal hormones as well as, thyroid hormones. The evolutionary trend seems to be to minimise the influence of such hormones on the reproductive axis as in the case of mammals or in those cases in which the influence persists as in the case of seasonally breeding mammals, the interactions of these hormones with reproductive axis has been made less complicated and more subtle.

The reproductive physiology of birds seems to offer a varitable picture indicating differential effects of prolactin, adrenal steroids and thyroid hormones on various facets of reproductive activities. It is quite likely that the relative importance of various hormones in the overall body physiology can vary in different species of birds, according to their geographic distribution and climatic factors and their adaptive strategy. Hence, it is not difficult to conceive variations in the modulating influence of different hormones on the reproductive functions of birds. This is well reflected in the many reports indicating parallel and inverse relationship between the adrenal steroids and thyroid hormones on one hand and gonadal hormones on the other hand (Thapliyal and Pandha, 1967; Chandola and Thapliyal, 1974; Jallageas and Assenmacher, 1974; Jallageas et al., 1978; Oishi and Konishi, 1978; Chaturvedi and Thapliyal,1980; Patel et al., 1985; Ramachandran and Patel, 1986). Even the role of prolactin has been shown to be different in different species of birds in terms of age, sex, reproductive status of birds etc(Meier, 1969; Meier et al., 1971; Stetson et al., 1973; Camper and Burke, 1977; Ensor, 1978; de Vlaming, 1979). The neuroendocrine regulation of reproductive functions in birds has projected a confusing picture mainly due to the investigation of individual endocrine glands and their hormones in isolation of others. Since seasonal reproduction requires an integration of the expected variations of the endocrine melieu in relation to circannual variations and environmental factors, a more objective understanding of the neuroendocrine regulation of reproductive functions in birds would be feasible only when investigations involving simultaneous study of various hormones in a single species are carried out on a seasonal basis. It is this line of thinking which has prompted investigations in this laboratory on interaction between various hormones on gonadal functions in feral pigeons on a seasonal basis.

Two of the endocrine glands reported to have modulating influence on seasonal reproductive functions in birds are the adrenals and thyroid. The fact that influences of such non-classical endocrine glands can vary greatly depending on the geographical distribution, climatic factors as well as the overall endocrine and metabolic strategy of birds,is well exemplified by the many divergent and contradictory results obtained by investigators to this date. A review of the literature to-date reveals both parallel and inverse relationships between these two endocrine glands and the gonads. Accordingly, Bhattacharya and Ghosh (1965), Moens and Coessens (1970), Smith and Brereton (1976), Thapliyal (1981) Patel et al.,(1985), Ramachandran and Patel (1986) and Ayyar et al.,(1992) have shown a parallel adrenal-gonad relationship in house sparrow, eastern rosella, lal munia & feral pigeons, while, Lorenzen and Farner (1964),Dusseau and Meier (1971)& Silverin (1979) have shown inverse adrenal-gonad relationship in white crowned sparrow, pied fly-catcher and the fowl. Similarly, Thapliyal and Chandola (1972), Hohn (1961), Van Tienhoven (1961), Assenmacher and Tixier vidal (1972), Thapliyal and Chaturvedi (1976), Jallageas and Assenmacher (1973), Chaturvedi and Thapliyal (1980), Thapliyal et al., (1982)& Ramachandran and Patel (1986), have shown parallel thyroid-gonad relationship in the canard, common myna, wild finch & domestic pigeons, while, Woitkewitsch (1940), Thapliyal and Pandha (1967),Thaliyal and Bageshwar (1970) Chandola and Thapliyal (1974), Oishi and Konishi (1978), showed inverse thyroid-gonad relationship in the lal munia, Indian weaver bird, starlings, Japanese quail and the feral pigeons.

A late but forceful entrant into the field is the pineal gland. Of the various functions known to be influenced by the pineal gland, are the cyclic ones like thermoregulation (see Ralph, 1978), colour change, locomotor activity and reproduction not to say many other circadian, circhoral and circannual changes (see Ralph, 1978, Gupta <u>et</u> <u>al</u>.,1987; see Underwood, 1989). One of the earliest recorded influence of pineal on the reproductive system in mammals was inhibition (Reiter <u>et</u> <u>al</u>.,1975; Johnson and Reiter, 1978; Bartness and Goldman, 1989). Over the years, this inhibitory influence got established in a wide variety of animal species and this action has now been ascribed to as the antigonadotrophic influence of its hormone melatonin. The pineal stands at the interface between environment and body functions, as a meuroendocrine transducer, helping to modulate the changes in the environmental cues. Since pineal essentially helps an animal to adapt to the external influences and adjust its intrinsic functions in an adaptive fashion, its influences observed under experimental conditions have been highly varied depending on the season, the time of the day as well as the dosage and duration of treatment with its hormone. In mammals, the influence of pineal has been found to be both profound & more inricate in seasonal breeders, as compared to the continuous breeders. The nocturnal output of melatonin by the pineal is dependent on the duration of the dark period and hence longer days lead to less melatonin production and shorter days to greater melatonin production and, it is this variations in melatonin production that triggers off the seasonal reproductive responses of the animal.

Apart from its influences on reproductive system , pineal and its indole hormone has also been known to have varied effects as can be gauged from the literature on adrenal and thyroid functions. Though by far the role pineal in the reproductive functions of mammals is getting more crystallized, its role in avian reproduction still remains enigmatic. Reports ranging from a progonadal action, or antigonadal action (Ramachandran et al., 1987; Ramachandran and Patel, 1988) or even both are all available. Some workers have even suggested no role of pineal in reproduction (Pitis et al., 1970; Ralph, 1978). Many factors such as different species, age, different time period and even a limited number of species studied have all contributed to this confusing and contradictory scenario. Its influences on adrenal and thyroid are not studied in birds unlike in mammals.

It is in this context, that detailed studies involving pineal, adrenal and thyroid on the seasonal male reproductive functions of a single species, the feral pigeons, Columba livia, were planned in this laboratory. Earlier studies on this line in this laboratory succeeded in recording a progonadal effect of the pineal, as PX in the recrudescent and breeding phases brought about testicular involution (Ramachandran et al., 1987; Ramachandran and Patel, 1988). A parallel adrenal-gonad relationship was also established as adrenocortical suppression in the breeding and recrudescent phases induced testicular involution. Observations revealed that, PX was also marked by adrenocortical involution, thereby alluding to a possible parallel pineal-adrenal relationship (Patel et al., 1985; Ramachandran and Patel, 1986) and, an inverse thyroid-gonad relationship was inferred from the observation of reduced thyroid activity in the breeding season and vice-versa in the non-breeding season (Patel et al.,1985; Ramachandran and Patel, 1986; Patel, 1993).An inverse pineal-thyroid interaction was also inferable as, PX in the recrudescent and breeding seasons increased the thyroid activity (Patel et al., 1985; Ramachandran and Patel 1986; Patel, 1993). All the above observation accrueing from the past studies suggested complex subtle interactions between pineal, adrenal and thyroid in the seasonal reproductive functions of sub-tropical feral pigeons. An understanding of these complex interactions in regulating the seasonal functions of testis needed complex experimental evaluation, involving manipulations of these endocrine glands. The current investigations, forming a part of this strategy, have helped elucidate the interactions of these glands better.

Though, parallel adrenal-gonad relationship has been reported in some species of birds (Bhattacharya and Ghosh, 1965; Moens and Coessoens, 1970; Smith and Brereton, 1976; Thapliyal, 1981), as well as in the case of tropical feral pigeons (Patel _et_ _al_.,1985; Patel and Ramachandran, 1986), the mechanosm of action of adrenal steroids on testicular functions has not been clearly elucidated. A concurrent evaluation of thyroid activity and function in birds subjected to adrenocortical suppression in the breeding season revealed an increased activity of thyroid. This is confirmed by histological observations showing colloidal depletion as well as elevated serum T_4 level (chapter I). Obviously, the testicular regression induced by adrenocortical suppression seemed to have some relationship with thyroid activity. In the wake of the many reports available indicating the ability of thyroxine (T_4) to bring about gonadal involution in some species of birds (Chandola and Bhatt,1982; Chandola _et_ _al_.,1982; Pathak and Chandola, 1983), the probable action of thyroxine in inducing testicular regression in feral pigeons as a consequence of adrenocortical suppression needed scrutiny. Interestingly, T_4 administration to pigeons in the breeding season could induce testicular regression (ongoing studies). These observations tend to suggest an influence of corticosteroids on the HHT axis. Though an inhibitory influence of corticosterone on HHT axis can be inferred, as marked by the increased colloid retention and decreased serum T_4 level in pigeons treated exogenously with corticosterone (CORT), this inhibitory action of CORT on the HHT axis seems to be independent of season, as this action of CORT on the HHT axis was observable in both breeding as well as non-breeding seasons. However, an interesting observation is that this action of CORT on HHT axis requires an intact pineal. This was confirmed by the observation that CORT was ineffective in inducing colloid retention and reducing serum T_4 level in PX pigeons. . It is this experimental treatment which gives the clue to the interrelationship between pineal, adrenal and thyroid in controlling testicular functions in the feral pigeon . Since PX induced testicular regression was already known to be paralled by adrenocortical regression and reduced serum CORT level (Patel et al.,1985; Ramachandran and Patel, 1986; Patel, 1993), it was natural to test whether, PX induced testicular regression could be reversed by CORT administration. It became evident that CORT administration was of no consequence in PX induced testicular regression (chapter I). Simultaneous evaluation of thyroid activity revealed no suppresive influence, as marked by neither colloid retention nor reduced serum T_4 level unlike in intact animals. It is presumable from these observations that, the sensitivity of the HHT axis to the inhibitory influence of CORT requires melatonin. Since it is shown that PX leads to colloid depletion and increase serum T_4 level and, melatonin administration to intact or PX pigeons leads to colloid retention and decrease in serum T_4 level (Patel,1993), an intact pineal by way of melatonin output can exert a suppressive influence on the HHT axis. These observations coupled with the inability of CORT to suppress the HHT axis in PX pigeons tend to suggest that the inhibitory influence of CORT on HHT axis in intact pigeons to be indirect. Obviously, the action of CORT on the HHT axis is manifested only in presence of melatonin and, presumably CORT essentially only potentiates the action of melatonin on the HHT axis. Apart from its influence on the HHT axis, melatonin also seems to have direct action on the HHA axis. This is inferable from the observed adrenocortical regression and decreased serum CORT level in PX pigeons and vice-versa in the melatonin treated ones (Patel,1993).

Overall, the detailed studies on the control of testicular functions in the tropical feral pigeons, seem to suggest increased adrenocortical activity coupled with reduced thyroid activity resulting in an increased CORT: T_4 ratio to be conducive for testicular functions. A reverse status is probably responsible for the quiescent state of the testes in the non-breeding season. However, the parallel adrenal-testis and an inverse thyroid-testis interactions are essentially due to the modulatory influence of melatonin on the HHT and HHA axis. In this scenario, melatonin emerges as a crucial factor in regulating the seasonal reproductive activity in male feral pigeons. A reasonable hypothesis that can be fashioned out, is that, the decreasing pineal melatonin output occurring during the gradually increasing longer days of summer months (April-May), on reaching a lower threshold level, looses its stimulatory action on the HHA axis and its inhibitory action on the HHT axis. This would lead to a decrease in CORT: T_4 ratio resulting in testicular regression. Similarly, the gradually increasing pineal melatonin output subsequent to the summer solstice on reaching a potentially higher threshold level during November-December, would re-establish its inhibitory influence on the HHT axis and a stimulatory influence on the HHA axis leading to increased serum CORT : T_4 ratio resulting in testicular recrudescence. Though an increased CORT : T_4 ratio is inferred favourable for testicular functions and, reduced CORT : T_4 ratio is characteristic of the non-breeding season,experimentally induced increased CORT : T_4 in the regression and non-breeding phases is incabable of inducing testicular functions. This is indicative of the fact that though an increased CORT :

T_4 ratio is favourable for testicular functions, the HHG axis is insensitive, once the testicular regression has been induced. The quiescent state of the testes in the non-breeding season is akin to a refractory state of the HHG axis as has been reported for many other species (Singh and Chandola, 1982; Maitra, 1987,a,b; Kumar and Kumar, 1991, 1992, 1993). Such an existence of a refractory state of the HHG axis is inferable not only from the observed inaibility of experimentally induced increased CORT : T_4 ratio to stimulate testicular functions but also from a previous report of an inability of increased photic schedule to induce testicular recrudescence in the month of November (Patel, 1993) inspite of an increase in CORT :T_4 ratio. However, testicular recrudescence was hastened by the exposure of pigeons to long photic schedule in the month of December (Patel, 1993). This indicates that the refractory state of the HHG axis is insensitive to the stimulatory influence of long photoperiod as well as the attendant increase in CORT :T_4 ratio. It remains to be tested as to whether the insensitivity of the HHG axis can be stimulated either by exposure of birds to a short photoperiod for a short duration followed by long photoperiod or experimental induction of low CORT : T_4 ratio, followed by an experimental induction of high CORT : T_4 ratio. Another inference drawn during the course of the present study is that experimentally induced increase in CORT: T_4 ratio is incapable of keeping the testis in the active state, once the testis has undergone regression in response to decreased CORT : T_4 ratio. Obviously, the ability of increase in CORT T_4 ratio to maintain testicular function can be gauged only if experimental induction of increased CORT: T_4 ratio is attempted earlier to a normally occurring testicular regression in the late breeding phase. One of the suggested explanations for the inactive state of the gonads, is its insensitivity or reduced sensitivity towards gonadotrophic hormones. In order to test this concept in the feral pigeons, they were treated with gonadotrophic hormones in the non-breeding phase. Since it was also shown that CORT has a favourable influence on testicular functions in the non-breeding phase (chapter II), the effect of prior CORT priming earlier to gonadotropin treatment as well as simultaneous administration of both was also assessed. Though CORT alone could induce some spermatogonial proliferation without activating spermatogenesis, both FSH and a combination of FSH.LH were able to initiate spermatogenesis. Obviously, the quiescence of the testes in the non-breeding phase could be broken by gonadotropin suggesting a lack of gonadotropin and not the insensitivity of the gonads, as the factor responsible.

Inspite of the fact that spermatogenesis was initiated under gonadotrophic treatment, the weight increase of the testes was not marked. This may be due to the very short period of treatment (15 days) which may not be sufficient to bring about dramatic testicular enlargement. Though, both FSH and FSH.LH treatment were marked by appearance of early meiotic cell types, the former induced significant hypertrophy of interstitium while the later induced proliferation of interstial cells. However, simultaneous treatment with CORT either in the form of priming or given concurrently with gonadotrophins proved to be more conducive for testicular recrudescence as the spermatogenic proliferation was both quantitatively and qualitatively better than with gonadotropins alone. Again, CORT + FSH appears to be more effective than CORT +FSH.LH. Due to the many conflicting reports regarding the relative importance of FSH or FSH.LH in the testicular physiology of birds (Jones, 1970; Brown et al., 1975;Murton and Westwood, 1977; Follet, 1978; Follett and Robinson, 1980;Sakai and Ishii; 1986; Peczely, 1989; Bluhm et al.,1991), the same question seems to arise for the feral pigeon as well. Apparently, the present observations tend to suggest FSH to be the dominant gonadotropin in testicular activation. Such an importance of FSH has also been reported in other avian species (Sakaii and Ishii, 1986). However, the competence of LH will have to be tested by treatment of birds with LH alone, which was not done in the present study. Moreover, the relatively less effectiveness of FSH.LH combination in the present study may also be due to the sub-optimal dosage, as the total dosage of FSH.LH was equal to that of FSH alone. These aspects need to be clearly clarified in future studies to arrive at a more prespective importance of the gonadotropins in the male feral pigeon . The dosages of the gonadotropins used in the present study are significantly less than those used by other workers (Lofts _et al_.,1973; see Murton & Westwood, 1977). Besides, the absence of dramatic increase in testicular weight as well as inability to establish spermatogenesis fully, seemed to be mainly due to the short duration of treatment. This is well understandable as the duration of spermatogenesis in most of the avian species is about 24-28 days. In this context, a long term treatment of atleast one month should be tried out to see its relative importance. To assess the qualitative or quantitative influences of corticosteroids on gonadotropin induced testicular activation, it would be also interesting to see the effect of treatment with gonadotrophin in pigeons rendered hypocorticalic. Since the breeding activities incur heavy energy expenditure, seasonal breeders have to reallocate annual budgetary provisions in terms of reproductive phases. Seasonal breeders can therefore be expected to show annual variatons in energy metabolism which must be under the purview of underlying alterations in the endocrine _milieu_. In this respect, importance of carbohydrates in energy metabolism of Indian feral pigeons in respect to testicular cyclicity has already been reported (Ayyar, 1987; Patel _et_ al.,1988; Patel, 1993). Besides, Pinealectomy (PX), and induced adrenocortical insufficiency have been shown to induce testicular regression in the breeding phase and concurrently alter plasma glucose level and tissue glycogen content (Ayyar, 1987; Ramachandran et al.,1987; Ramachandran and Patel, 1986; Patel et al.,1988). In order to gain a proper prespective on circannual variation in carbohydrate metabolism, the content of glycogen in liver, muscle and testis and blood glucose and activity levels of phosphorylase and G-6-P'ase has been evaluated on a monthly basis, Besides, the effects of PX as well as exogenous CORT and gonadotrophins on carbohydrate metabolism have been assessed in specific seasons. The monthly changes in the various parameters suggest the recrudescent and breeding phases to be energetically demanding as marked by depletion of tissue carbohydrate reserve and maintainence of high blood glucose level. The observation also tend to highlight muscle glycogen as an important source of energy during the late non-breeding to the pre-recrudescent phases. These changes in carbohydrate metabolism seem to have some relation with circannual changes in adrenocortical activity as depletion of tissue glycogen stores and hyperglycemia occurring in the recrudescent and breeding phases correspond to higher adrenocortical activity and serum CORT level and, increase in tissue glycogen content and low blood glucose level occurring during the non-breeding season, correspond to decreased adrenocortical activity and lower serum CORT level. Obviously, the parallel adrenal-testes relationship seems to have a direct bearing on carbohydrate utilization and energy production. This was confirmed by the observation of increased tissue glycogen content and decreased blood glucose level in the breeding season in pigeons rendered hypocorticalic and a reverse set of changes in pigeons rendered hypercorticalic in regression and non-breeding phases. Though CORT administration to pigeons in the regression and non-breeding phases could alter carbohydrate metabolism to a more characteristic recrudescent and breeding specific pattern, it was nevertheless without any effect in inducing testicular activation. This is most likely due to the insensitivity of the HHG axis to CORT subsequent to testicular regression. It has also been revealed that the characteristic effects of CORT on carbohydrate metabolism on a season specific fashion require an intact pineal as CORT administration to PX pigeons either in the regression or non-breeding phases was unsuccessful in inducing the characteristic alteration in carbohydrate metabolism as observed in intact birds. In fact, CORT administration to PX pigeons tended to potentiate the PX effects marked by tissue glycogen depletion and hypoglycemia. These changes induced by PX are independent effects, related to decreased hepatic glucose uptake increased peripheral utilization, increased insulin release/sensitivity (Patel and Ramachandran, 1992). Inferably, CORT influence on carbohydrate metabolism in intact and PX pigeons is differential and the characteristic action of CORT seems to be occurring on a background of an optimum melatonin level. It would be interesting to see in this context as to whether simultaneous treatment of PX pigeons with CORT and melatonin would be able to produce the characteristic changes in carbohydrate metabolism as observable in intact pigeons. The influence of gonadotrophins on carbohydrate metabolism in the non-breeding season was thought pertinent as gonadotrophins were noted to increase the adrenocortical activity. In keeping with this, FSH.LH was able to induce characteristic breeding specific effects on carbohydrate metabolism such as tissue glycogen depletion and hyperglycemia. A common effect of tissue glycogen depletion by either FSH alone or a combination of FSH.LH with or without CORT is compatible with the induced testicular recrudescence and increased adrenocortical activity. However, FSH or, CORT+FSH induced hypoglycemia, rather than hyperglycemia. This effect on the glycemic status is opposite to that of CORT or FSH.LH. A plausible explanation in this context is that while, FSH might potentiate peripheral utilization of glucose either directly or through insulin and also nullify the CORT effect on peripheral utilization, the hyperglycemic state in CORT or FSH.LH could be due to hyperphagia as well as decreased peripheral uptake due to insulin resistance. The ability of CORT to induce insulin resistance and hyperphagia is a reported fact (Nagra and Meyer, 1963; Taouis et al., 1993). Moreover, a previous study from this laboratory demonstrated increased insulin sensitivity in chicks rendered hypocorticalic (John, 1990). The present investigations have shown an interesting relationship between gonadotrophins and carbohydrate metabolism. Since there are no other reports in scientific literature on these lines, more searching scrutiny on this aspect is warranted.

The evaluation of circannual variations in tissue ascorbic acid (AA) content and the influences of CORT on the same in different reproductive phases in intact and PX pigeons have revealed a positive relationship between pineal, adrenal and AA content. In general, there is a positive correlation between CORT and AA content as, during the recrudescent and the breeding season~~s~~ both were increased and, a parallel decrease in the CORT level and AA content occurred in the non-breeding season. This is substantiated by the observation that both PX as well as hypocorticalism in the breeding season decreased tissue AA content while CORT administration in the quiescent phase increased the AA content. The decreased AA content in the testis during the breeding season, when the hepatic and adrenal AA are very high, is correlatable with the depletion of AA due to increased steroidogenic and spermatogenic activity. Substantial support to this evidence is available for the ability of CORT to induce a positive AA balance from the previous works in this laboratory as well as from others (Stewart _et al._,1953; Giovanni _et al._,1957; Cuzzocrea _et al._,1959; Sinha and Lahiri, 1964; Joseph and Ramachandran, 1991). An interesting revelation from this study is a differential season specific sensitivity of the hypothalamic feedback center to CORT. The current observations tend to indicate a lowered sensitivity of the feedback center to CORT starting from the quiescent phase and extending upto the breeding phase resulting in a probable positive feedback action of CORT on ACTH secretion. This is marked by the continous increase in the adrenal AA content and serum CORT level throughout the recrudescent and breeding phases. Ultimately, a high threshold level of CORT attained towards the end of breeding season seems to sensitize the negative feedback center leading to a sudden decrement in adrenal AA content and serum CORT level and concomitant testicular regression. This contention is supported by the observed positive influence of CORT on the adrenal AA in the quiescent phase. Evaluation of tissue AA content in gonadotropin treated pigeons in the quiescent phase has shown the ability of these hoemones to induce positive AA balance when administered either alone or in combination with CORT. This action was however, more potent in FSH treated birds than in birds treated with a combination of FSH.LH. Overall, the observations indicate a favourable influence of CORT or FSH or more potently a combination of the two to induce a positive AA balance and promote mobilization of AA by the adrenals and testis, while LH seems to have a preferential influence on utilization. In a hypothelical sense, it is concluded that, during normal testicular recrudescence, an initial increase in FSH in combination with CORT activates the testis as well as induces AA mobilization and subsequent decrement in FSH coupled with an increment in LH might lead to AA utilization and increased steroidogenesis.

Overall, the present investigations have helped elucidate the subtle interactions between pineal and adrenal in testicular functions as well as metabolic activities as well as, thrown up many hitherto unknown grey areas which would need scrutiny and further experimental evaluations, providing challenging avenues for furture investigations.